

# Evaluation of immunological markers among women with recurrent abortion in Thi-Qar province

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## SUMMARY

**AUTHORS' CONTRIBUTION:** (A) Study Design · (B) Data Collection · (C) Statistical Analysis · (D) Data Interpretation · (E) Manuscript Preparation · (F) Literature Search · (G) No Fund Collection

Many women around the world suffer from recurrent miscarriages associated with immune disorders leading to decreased maternal and fetal tolerance. Thus, this study designed to evaluate the levels of Antiphospholipid antibodies (APL IgG & IgM and TGF-β1 in patients' women with recurrent abortions by using Enzyme-Linked Immune Sorbent Assay (ELISA). The current study included 100 women blood samples with recurrent miscarriage and 50 blood samples from apparently healthy women as control group their ages ranged between 17- 45 years. The results of the statistical analysis showed a significant increase in the levels of IgM, IgG and TGF-β1 in patients with recurrent miscarriage compared to the control group. Furthermore, highly significant positive strong correlation between IgM and IgG APL antibodies, and non-significant weak positive correlation between TGF-β and both antibodies (IgM and IgG APL) were indicated. In conclusion, TGF-β1 levels in miscarriage women are closely related to APLA levels during pregnancy and may have an influential role in pregnancy outcomes.

**Keywords:** Recurrent miscarriage; Antiphospholipid; TGF- β1 cytokine; ELISA

## INTRODUCTION

Miscarriage is defined as the spontaneous loss of a pregnancy before the fetus reaches life, and as such includes all pregnancy losses from conception through 24 weeks of gestation [1].

Recurrent miscarriage, the spontaneous loss of two or more consecutive intra-uterine pregnancies with the same partner before 20 weeks' gestation, is known to affect 0.5–2% of pregnant women [2]. It has been suggested that the causes are multi-factorial and might include parental chromosomal abnormalities, maternal thrombophilia, immune dysfunction and endocrine disorders [3]. Miscarriage is a common and distressing complication of early pregnancy whatever gestation it occurs at and whether it meets the terminology of a biochemical pregnancy or a clinical miscarriage [4]. An estimated 23 million miscarriages occur each year worldwide, which equates to 44 miscarriages every minute [5].

Antiphospholipid Syndrome (APS) is the most frequently diagnosed immunological cause of Recurrent Spontaneous Miscarriage (RSM) and the only proven thrombophilia associated with an adverse pregnancy outcome, and has also been identified as the most treatable cause of RSM [6]. APL is also associated with other symptoms, Obstetric complications, such as preeclampsia, intrauterine growth restriction, and prematurity [7]. Previous study indicated that Antiphospholipid antibodies are an important cause of mid-trimester recurrent abortion in Iraqi women, with intermediate frequencies between Western and Indian reports [8].

Transforming Growth Factor –Beta1 (TGF-β1) is a pleiotropic cytokine that functions in tissue fibrosis, wound healing, and embryonic development [9]. It stimulates the production of Regulatory T cells (Tregs), maintain and mediate the immunosuppressive function of Tregs cells, the balance of M1/M2 macrophage, also has a role in regulation function of natural killer cell. Thus, it is maintaining the immune tolerance of the fetus and mother.

In addition to some studies showed that TGF-β1 is dysregulated in patients with recurrent spontaneous abortion or preeclampsia [10].

Based on what previous studies marked about the relationship of antiphospholipid antibodies to recurrent miscarriage and the role that TGF-β plays in maintaining pregnancy and preventing miscarriage, this study was

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designed to evaluate the levels of antiphospholipid antibodies (APL IgG, IgM,) and TGF- $\beta$  antibodies in women with recurrent miscarriage in Thi Qar Governorate.

## MATERIALS AND METHODS

### Sample collection

This study conducted in Labs of Collage of Education for Pure Science/Thi-Qar university in collaboration with of Bunt Al-Huda Hospital Teaching Hospital, Thi-Qar, Iraq during the period from 1st of February 2023 to 31<sup>st</sup> August 2023.

Overall, 150 people were enrolled in this study, one hundred of whom were women with recurrent miscarriage who were divided into three groups according to age, and fifty healthy women as a control group, aged 17- 45 years. All participating women were evaluated for specific antiphospholipid antibodies IgM, IgG, and TGF- $\beta$ 1 using enzyme-linked immunosorbent assay ELISA (Biotic, USA)

### Statistical analysis

The statistical analysis program SPSS was used to analyze the results of the current study, where the independent student t-test was used to compare means between two groups, and Person's correlation coefficient test that measured linear correlation between studied parameters at (P. value <0.05, P. value <0.01)

## RESULTS

**Tab.1.** showed the value of Immunological Parameters results in miscarriages women and healthy women control group. The current findings showed that women with recurrent miscarriage abortion had an increased serum level of all involved immunological parameters, compared with the healthy women with significant difference at p. value <0.05.

Regarding to the APL antibodies results, the current study showed highly significant increase ( $P \leq 0.05$ ) in the concentration of both types of antiphospholipid antibodies (IgM, IgG) in serum of patients women group with miscarriage abortion compared to a healthy control group, and APL (IgM) appeared higher concentration than APL (IgG).

According to Person's correlation coefficient between study parameters the present results indicated highly significant positive strong correlation between IgM and IgG APL antibodies, while non-significant weak positive correlation between TGF- $\beta$  and both antibodies (IgM and IgG APL) as in **Tab. 2.**

## DISCUSSION

These results are consistent with the results of a local study in Thi Qar conducted by Hamadi and Lafta, which indicated increased APA IgG/IgM in patients with recurrent miscarriage compared with the control group. They also found that the positive rates of APL (IgM) were higher than APL (IgG) [11].

Also, the same results were confirmed by Ilyas, et al. who found that the percentages of positivity for APL (IgM) are higher than for APL (IgG) [12].

This an elevated levels of IgM antibodies may be due to the transient and non-specific nature of these antibodies, where increase secondarily after exposure to drugs and infections. Whereas antiphospholipid IgG antibodies are more specific and are commonly associated with thrombosis, thrombocytopenia, and recurrent miscarriage [13].

The high results of these ApLs were directly involved in thrombosis that leading to placental infarction and fetal death, a previous study indicated that antibodies against APL target the placenta and stimulate platelet and endothelial cell activation, leading to a prothrombotic state. But all this is not enough to cause fetal loss or delayed growth. Some minor mediators such as C3a, C5a and C5b-9 MAC are produced by complement activation leading to inflammation, tissue damage and finally fetal loss *via* enhancement of platelets and endothelium Cell activation [14].

Moreover, the current results showed an increase in TGF- $\beta$ 1 mean concentration levels among the group of patient women compared to the healthy pregnant control group, Table 1. These results are consistent with two previous Iraqi studies that indicated a significant increase in the mean levels of the anti-inflammatory marker (TGF- $\beta$ ) in women with recurrent miscarriage compared to healthy pregnant women [15,16]. This is also in line with Ogasawara, et al. who found that TGF-beta levels among pregnant women

**Tab. 1.** Sera levels of immunological parameters in patients women and healthy groups.

Immunological Parameters	Patients	Control	T test P value
	Mean $\pm$ S. D		
TGF- $\beta$ 1	7.64 $\pm$ 2.79	6.27 $\pm$ 2.14	< 0.01**
APL IgM	0.80 $\pm$ 0.39	0.64 $\pm$ 0.17	< 0.01**
APL IgG	0.39 $\pm$ 0.13	0.25 $\pm$ 0.07	< 0.01**

**Tab. 2.** Person correlation between immunological parameters.

Person		IgM	IgG
TGF- $\beta$ 1	r	0.050	0.120
	p. value	0.622	0.233
IgM	r	-	0.702**
	p. value	-	0.00

who had miscarriages were significantly higher than among control pregnant women, and concluded that TGF-beta1 may be important for maintaining pregnancy but may also be a risk factor for recurrent miscarriage [17].

While the current findings contradicts the results of a recent study that showed significantly higher levels for TFG- in women without a history of recurrent miscarriage compared to women with a history of recurrent miscarriage [18]. Huber, et al. indicated that the serum TGF-beta1 levels, does not appear to have a role in the pathogenesis of preeclampsia [19].

These differences among the results of studies may be due to variety of the time and place of sample collection, the techniques and kits used for diagnosis, and the size or types of samples tested.

TGF-β has a cardinal role in maintaining peripheral tolerance to self and harmless antigens, such as fetal alloantigens [10, 20].

Previous data published by Magdoud and his colleagues provided the first evidence on the effect of TGF-β genotype in spontaneous abortion, and they indicated the important role that TGF-β plays in pregnancy outcomes [21].

Antiphospholipid antibodies are acquired autoantibodies against phospholipids that linked with slow progressive thrombosis and placental infarction.

Phospholipids present in the blood play an important role in the blood clotting process. In some people, the body mistakenly identifies phospholipids as foreign substances and forms antibodies against them. As a result of confusion in the immune system, which leads to the autoimmune disorder [22].

Many studies indicated that the levels of both antibodies (IgM and IgG APL) increased in patients with recurrent miscarriage [11-12].

Jakiela, et al. founded that the TGF- β1 production were decreased in primary antiphospholipid syndrome PAPS patients with high levels of aPL IgG [22].

## CONCLUSION

The study concluded that significantly elevated TGF-β1 levels in recurrent aborted women are closely related to APLA levels during pregnancy and may have an influential role in pregnancy outcomes.

## DISCLOSURE

None.

## FUNDING

None.

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